Amendments to the Specification:

On page 1 of the specification, please amend the title to read as follows:

"*Helicobacter Pylori* CAI Antigen."

Please insert the following paragraph on page 1 following the title:

--This application is a divisional of U.S. Application Serial No. 09/410,835, filed October 1, 1999, which is a continuation of U.S. Application Serial No. 08/471,491, filed June 6, 1995, now issued as U.S. Patent No. 6,090,611, which is a divisional application of U.S. Application Serial No. 08/256,848, filed October 21, 1994, now abandoned, which is a U.S. national phase application of PCT/EP93/00472, filed March 2, 1993 and PCT/EP93/00158, filed January 25, 1993, which two PCT applications claimed priority benefit of Italian Application Serial No. FI 92 A 000052, filed March 2, 1992, the entire contents of each application is incorporated in its entirety by reference herein.--

Please amend the paragraph bridging pages 3-4 as follows:

--The present invention The present invention describes nucleotide and amino acid sequences for three major ~~H. pylori~~ *H. pylori* proteins. Specifically, these are the cytotoxin, the "Cytotoxin Associated Immunodominant" (CAI) antigen, and the heat shock protein. None of the complete amino acid sequences for these proteins has been known, nor have their genes been identified. The present invention pertains to not only these purified proteins and their genes, but also recombinant materials associated therewith, such as vectors and host cells. The present invention provides cytotoxin polypeptides that exhibit substantially no toxicity, or substantially reduced toxicity. The present invention also provides CAI and heat shock polypeptides that exhibit no functional contribution to toxicity, or a substantially reduced functional contribution to toxicity. The understanding at the molecular level of the

nature and the role of these proteins and the availability of recombinant production has important implications for the development of new diagnostic for ~~H. pylori~~ *H. pylori* and for the design of vaccines that may prevent ~~H. pylori~~ *H. pylori* infection and treat disease.--

Please amend the paragraph at page 4, lines 26-27, as follows:

-- ~~Fig. 1 is~~ Figs. 1A, 1B, and 1C (SEQ ID NO:2) comprise the nucleotide sequence for the cytotoxin (CT) protein.--

Please amend the paragraph at page 4, lines 28-29, as follows:

-- Fig. 2 is the amino acid sequence for the cytotoxin (CT) protein (SEQ ID NO:3).--

Please amend the paragraph at page 4, lines 33-34, as follows:

-- ~~Fig. 4 is~~ Figs. 4A-4F comprise the nucleotide (SEQ ID NO:4) and amino acid (SEQ ID NO:5) sequences of the CAI antigen. The numbers along the left-hand margins of Figs. 4A, 4C, and 4E designate amino acid position, and the numbers along the right-hand margins of Figs. 4B, 4D, and 4F designate the nucleotide positions.--

Please amend the paragraph at page 4, lines 35-36, as follows:

-- ~~Fig. 5 is~~ Figs. 5A, 5B, and 5C comprise the nucleotide (SEQ ID NO:7) and amino acid (SEQ ID NO:6) sequences of the heat shock protein (hsp).--

Please amend the paragraph bridging pages 39-40 as follows:

--Preferred adjuvants to enhance effectiveness of the composition include, but are not limited to: (1) aluminum salts (alum), such as aluminum hydroxide, aluminum phosphate,

aluminum sulfate, *etc.*; (2) oil-in-water emulsion formulations (with or without other specific immunostimulating agents such as muramyl peptides (see below) or bacterial cell wall components), such as for example (a) MF59 (PCT Publ. No. WO 90/14837), containing 5% Squalene™, 0.5% Tween 80™, and 0.5% Span 85™ (optionally containing various amounts of MTP-PE (see below), although not required) formulated into submicron particles using a microfluidizer such as Model 110Y microfluidizer (Microfluidics, Newton, MA), (b) SAF, containing 10% Squalane, 0.4% Tween 80™, 5% pluronic-blocked polymer L121, and thr-MDP (see below) either microfluidized into a submicron emulsion or vortexed to generate a larger particle size emulsion, and (c) Ribi™ adjuvant system (RAS), (Ribi Immunochem, Hamilton, MT) containing 2% Squalene™, 0.2% Tween 80™, and one or more bacterial cell wall components from the group consisting of monophosphorylipid A (MPL), trehalose dimycolate (TDM), and cell wall skeleton (CWS), preferably MPL + CWS (Detox™); (3) saponin adjuvants, such as Stimulon™ (Cambridge Bioscience, Worcester, MA) may be used or particles generated therefrom such as ISCOMs (immunostimulating complexes); (4) Complete Freunds Adjuvant (CFA) and Incomplete Freunds Adjuvant (IFA); (5) cytokines, such as interleukins (IL-1, IL-2, etc.), macrophage colony stimulating factor (M-CFS), tumor necrosis factor (TNF), *etc.*; and (6) other substances that act as immunostimulating agents to enhance the effectiveness of the composition. Alum and MF59 are preferred.--

Please amend the paragraph spanning pages 49-50 as follows:

-- DNA manipulation was performed using standard procedures. DNA sequencing was performed using Sequenase 2.0 (USB) and the DNA fragments shown in Fig. 3 subcloned in Bluscript KS+. Each strand was sequenced at least three times. The region between nucleotides 1533 and 2289, for which a DNA clone was not available, was amplified

by PCR and sequenced using asymmetric PCR, and direct sequencing of amplified products. The overlapping of this region~~,~~ was confirmed by one- and double-side anchored PCR: an external universal anchor (5'-GCAAGCTTATCGATGTCGACTCGAGCT-3' (SEQ ID NO:1)/ 5'-GACTCGAGTCGACATCGA-3' (SEQ ID NO:8)) containing a protruding 5' HindIII sequence, and the recognition sites of ClaI, SalI, XhoI, was ligated to primer-extended DNA and amplified. A second round of PCR using nested primers was then used to obtain fragments of DNA suitable for cloning and sequencing. DNA sequence data were assembled and analyzed with the GCG package (Genetics Computer Group, Inc., Madison, WI) running on a VAX 3900 under VMS. The GenBank and EMBL databases were examined using the EMBL VAXcluster.--

Please amend the paragraph spanning pages 52-53 as follows:

--The ~~cai~~ *cai* gene coded for a putative protein of 1147 amino acids, with predicted molecular weight of 128012.73 Daltons and an isoelectric point of 9.72. The basic properties of the purified protein were confirmed by two dimensional gel electrophoresis. The codon usage and the GC content (37%) of the gene were similar to that described for other ~~H. pylori~~ *H. pylori* genes (13, 26). A putative ribosome binding site: AGGAG, was identified 5 base pairs upstream from the proposed ATG starting codon. Computer search for promoter sequences of the region upstream from the ATG start codon, identified sequences resembling either -10 or -35 regions, however, a region with good consensus to an ~~E. coli~~ *E. coli* promoter, or resembling published ~~H. pylori~~ *H. pylori* promoter sequences was not found. Primer extension analysis of purified ~~H. pylori~~ *H. pylori* RNA showed that 104 and 214 base pairs upstream from the ATG start codon there are two transcriptional starts sites. Canonical promoters could not be identified upstream from either transcriptional initiation sites. The

expression of a portion of the CAI antigen by clone 57/D suggests that ~~E. coli~~ *E. coli* is also recognizing a promoter in this region, however, it is not clear whether ~~E. coli~~ *E. coli* recognizes the same promoters of ~~H. pylori~~ *H. pylori* or whether the ~~H. pylori~~ *H. pylori* DNA that is rich in A-T provides ~~E. coli~~ *E. coli* with regions that may act as promoters. A rho independent terminator was identified downstream from the stop codon. In Fig. 4, the AGGAG ribosome binding site and terminator are underlined, and the repeated sequence and motif containing 6 asparagines are boxed. The CAI antigen was very hydrophilic, and did not show obvious leader peptide or transmembrane sequences. The most hydrophilic region was from amino acids 600 to 900, where also a number of unusual features can be observed: the repetition of the sequences EFKNGKNKDFSK (SEQ ID NO:9) and ~~EPYIA~~ EPIYA (SEQ ID NO:10), and the presence of a stretch of six contiguous asparagines (boxed in Fig. 4).--

Please amend the paragraph at page 61, lines 5-29 as follows:

-- The purified fusion protein was tested by Western blot using sera of patients infected by *H. pylori* and affected by atrophic and superficial gastritis, and patients with duodenal and gastric ulcers: most of the sera recognized the recombinant protein. However, the degree of recognition greatly varied between different individuals and the antibody levels did not show any obvious correlation with the type of disease. In addition, antibodies against *H. pylori* antigens and in particular against hsp protein were found in most of the 12 sera of patients affected by gastric carcinoma that were tested. Although *H .pylori* hsp recognition could not be put in relation with a particular clinical state of the disease given the high conservation between *H. pylori* hsp and its human homolog, it is possible that this protein may induce autoimmune antibodies cross-reacting with the human counterpart. This class of homologous proteins has been implicated in the induction of autoimmune disorders in

different systems. ~~Then present~~ The presence of high titers of ~~anti-H. pylor~~ anti-*H. pylori* hsp antibodies, potentially cross-reacting with the human homolog in dyspeptic patients, suggests that this protein has a role in gastroduodenal disease. This autoreactivity could play a role in the tissue damage that occurs in ~~H. pylori~~ *H. pylori*-induced gastritis, thus increasing the pathogenic mechanisms involved in the infection of this bacterium.--

Please amend the first paragraph on page 61 of the specification as follows:

--The following materials were deposited on December 15, 1992 and January 22, 1993 by Bioscine Sclavo, S.p.A.~~, the assignee of the present invention,~~ with the American Type Culture Collection (ATCC), 10801 University Boulevard, Manassas, VA 20110-2209 ~~12301 Parklawn Drive, Rockville, MD, phone (301) 231-5519~~, under the terms of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for Purposes of Patent Procedure. For the cytotoxin protein (CT):

ATCC No.: 69157 *E. coli* TG1 containing the plasmid TOXHH1

ATCC No.: n/a *E. coli* TG1 containing the plasmid TOXEE1

For the CAI protein:

ATCC No. 69158 *E. coli* TG1 containing the plasmid 57/D

ATCC No. 69159 *E. coli* TG1 containing the plasmid 64/4

ATCC No. 69160 *E. coli* TG1 containing the plasmid P1-24

ATCC No. 69161 *E. coli* TG1 containing the plasmid B/1

For the heat shock protein (hsp):

ATCC No. 69155 *E. coli* TG1 containing the plasmid pHp60G2

ATCC No. 69156 *E. coli* TG1 containing the plasmid pHp605.--